Identification and regulation of (novel) human adipokines : a proteomic approach

Summary

Obesity and the obesity-associated metabolic dysfunctions are a major health and economic burden. Adipocyte-secreted proteins play an important role in obesity-associated disorders. Therefore it is essential to study the secreted factors of human (pre)adipocytes, not only to understand the underlying mechanisms of obesity-induced metabolic complications, but also to gain more insight in the regulation of weight gain, weight loss, weight re-gain and weight maintenance. This may aid the development of successful treatment strategies for obesity and its related complications.

In vivo studies are usually confined to plasma and other body fluids and as such the results demonstrate the effect of an intervention but not the underlying molecular mechanisms. That is why *in vitro* studies of human (pre)adipocytes and adipose tissue are necessary. Technical issues of proteomics technologies, including false positive identification, limited throughput, low sensitivity and quantification still do not allow identifying the complete (pre)adipocyte secretome. However, improved MS approaches developed during the last 10 years have helped to increase the understanding of the adipose tissue/adipocyte proteome and its regulation, including the adipocyte-secreted proteins. Nevertheless, much is still unknown about the molecular mechanisms responsible for the development of obesity and its metabolic complications, as well as strategies to improve the obesity-induced metabolic phenotype. This thesis describes *in vitro* studies with human SGBS (pre)adipocytes which were investigated regarding the regulation of (pre)adipocyte-specific secreted proteins in the context of triglyceride (TG) accumulation/excess, adipocyte differentiation and TG reduction/increased lipolysis.

Chapter 2 evaluates relevant proteomic technologies in the field of adipocyte biology. In this context new findings, new molecular aspects of adipocyte biology and the discovery of novel adipokines within the adipocyte research field were presented and discussed. They have led to the conclusion that a combination of analysis techniques is essential to cover the identification of the total (pre)adipocyte proteome as well as changes therein.

Secretion differences of SGBS (pre)adipocytes during adipogenesis are described in **chapter 3**. Secretome profiling was performed on both cell types which led to the identification of 6 novel human adipocyte-secreted proteins. In addition, this study revealed 20 proteins that had not been detected before in human adipose material. In addition, 23 proteins that were previously detected in visceral adipose tissue were found to be secreted by SGBS-cells of subcutaneous origin. Furthermore, this secretome study confirmed SGBS cells as an ideal human (pre)adipocyte cell strain for adipocyte-related proteomics studies.

Tissue factor (TF) is involved in blood coagulation. Consequently, altered expression of TF during adipose tissue expansion might contribute to the development of obesity-induced thrombosis. As such, we investigated possible TF expression by SGBS (pre)adipocytes, which is reported in **chapter 4**. On both cell types and in the medium TF-mediated factor Xa and thrombin activation was observed. Our results indicate that human (pre)adipocytes express functional TF, which may have implications for obesity-induced thrombosis but also for extravascular fibrosis and angiogenesis.

Increased TG accumulation leads to increased adipose tissue mass, which might result in hypoxic conditions. Hypoxic areas have been observed in WAT of obese persons. This may lead to WAT dysregulation and development of obesity-associated metabolic dysregulation. In **chapter 5** we describe that $CoCl_2$-induced hypoxic secretome changes of (pre)adipocytes are mostly associated with protein down-regulation and a dysregulation of the extracellular matrix. This confirmed the hypothesis that hypoxia induces detrimental effects within enlarged adipose tissue of obese subjects. Such dysregulation was reflected by an up-regulation of collagens in adipocytes which indicated a cell survival process. In preadipocytes collagens were down-regulated which might be indicative for a disturbed differentiation process. In addition, we identified 9 novel (pre)adipocyte secreted proteins of which 6 were regulated by hypoxia.

Treatment strategies for obesity and obesity-induced complications are essential to cope with their associated medical problems. As such, investigation of promising drugs or food supplements is a key feature of obesity research. Since resveratrol (RSV) is known to mimic beneficial health effects of calorie restriction more and more research is performed to understand its biological mechanism. The study in **chapter 6** reveals an RSV-mediated increase of intracellular lipolysis, which resulted in a beneficial change of the adipocyte secretion profile. As such, ECM proteins were down-regulated while process-related proteins were mostly up-regulated. In addition, the secretion of proteins that are protective against cellular stress and of proteins involved in the regulation of apoptosis were up-regulated. Furthermore, RSV induced an up-regulation of adiponectin and ApoE and a down-regulation of PAI-1 and PEDF secretion. This indicated a positive impact on the adipocyte-secretome towards an improved obesity-associated inflammatory phenotype and metabolic dysfunction. In addition, 2 novel RSV-regulated adipocyte-secreted proteins were identified.

Another treatment strategy of obesity is a calorie restricted diet. An *in vitro* effect of CR on the human adipocyte secretion profile had not been investigated before. As such, **chapter 7** describes the identification of 6 novel adipocyte-secreted proteins that are regulated by CR.

In addition, it was shown that CR-induced adipocyte TG reduction led to a positively affected adipokine secretion pattern indicative for an improved inflammatory phenotype and an improved obesity-associated metabolic dysfunction including insulin resistance and glucose intolerance.
To distinguish between advantages and disadvantages of an RSV versus a calorie restricted intervention a comparison of the secretion profile of both obesity treatment strategies was also performed and described in **chapter 7**. CR and RSV differently affected the SGBS adipocyte secretion profile in which both interventions show an improvement of the obesity-associated inflammatory phenotype and the obesity-associated metabolic disorders while CR seems less forceful but appears to reach its positive effects with minor cellular stress.

All these studies were related to the investigation of the human (pre)adipocyte secretome. In **chapter 8** the results have been critically discussed in the context of the advantages and disadvantages of the used human cell model and the applied proteomics techniques. It is argued that technical improvement of analysis methods together with the acceptance of individual biological responses might increase the understanding of the endocrine function of the adipose tissue. This may aid the further development of improved obesity treatment strategies.